

WHAT IS CLAIMED IS:

1 1. A method for mapping a site of post-translational modification on a
2 post-translationally modified polypeptide, said method comprising:

3 (a) site-specifically cleaving a peptide bond of the post-translationally
4 modified polypeptide with an endopeptidase at said site of post-translational modification to
5 produce a degraded post-translationally modified polypeptide; and

6 (b) after step (a), determining said site of post-translational modification.

1 2. The method of claim 1, wherein said post-translational modification is
2 selected from phosphorylation, sulfonation, glycosylation, acetylation, methylations, ADP-
3 ribosylation, methionine oxidation, cysteine oxidation, and cysteine lipidation.

1 3. The method of claim 1, wherein said post-translational modification is
2 phosphorylation of an amino acid selected from tyrosine, serine, and threonine.

1 4. The method of claim 1, wherein said post-translational modification is
2 sulfonation of a tyrosine.

1 5. The method of claim 1, wherein said site of post-translational
2 modification is determined by a method comprising determining the mass spectrometry
3 fragmentation pattern of the degraded post-translationally modified polypeptide.

1 6. The method of claim 1, wherein said endopeptidase is a serine protease
2 comprising an active site that specifically binds to said post-translational modification.

1 7. The method of claim 6, wherein said serine protease is subtilisin.

1 8. A serine protease which site-specifically cleaves a peptide bond of a
2 post-translationally modified polypeptide at a site of post-translational modification, wherein
3 said serine protease comprises an active site that binds to said site of post-translational
4 modification.

1 9. The serine protease of claim 8, wherein said post-translational
2 modification is selected from phosphorylation, sulfonation, glycosylation, and acetylation.

1 10. The serine protease of claim 8, wherein said post-translational
2 modification is phosphorylation of an amino acid selected from tyrosine, serine, and
3 threonine.

1 11. The serine protease of claim 8, wherein said post-translational
2 modification is sulfonation of a tyrosine.

1 12. The serine protease of claim 8, wherein said serine protease is
2 subtilisin.

1 13. The serine protease of claim 8, wherein said serine protease is encoded
2 by a nucleic acid sequence that hybridizes under highly stringent hybridization conditions to
3 a nucleic acid encoding a polypeptide comprising an amino acid sequence of Figure 1,
4 wherein the hybridization reaction is incubated at 42°C in a solution comprising 50%
5 formamide, 5x SSC and 1% SDS, and washed at 65°C in a solution comprising 0.2x SSC and
6 0.1% SDS.

1 14. The serine protease of claim 8, wherein said serine protease comprises
2 a subsequence having at least 70% amino acid sequence identity to an amino acid sequence
3 of Figure 1.

1 15. The serine protease of claim 8, wherein said serine protease comprises
2 a subsequence having at least 70% amino acid sequence identity to an amino acid sequence
3 of Figure 1 and contains at least one amino acid substitution selected from P129G, E156R,
4 S191K, G166K, and G127S.

1 16. The serine protease of claim 8, wherein said serine protease is encoded
2 by an expression vector.

1 17. A host cell comprising the expression vector of claim 16.

1 18. An endopeptidase that site-specifically cleaves a peptide bond of a
2 post-translationally modified polypeptide at a site of post-translational modification, said
3 endopeptidase produced by a method comprising:
4 (a) introducing one or more point mutations to a model endopeptidase at one
5 or more candidate amino acid positions in an active site of said model endopeptidase to

6 produce a plurality of candidate endopeptidases, wherein at least one of said plurality of
7 candidate endopeptidases is an endopeptidase that site-specifically cleaves a peptide bond of
8 a post-translationally modified polypeptide at a site of post-translational modification; and
9 (b) identifying said endopeptidase that site-specifically cleaves at said site of post-translational
10 modification.

1 19. The endopeptidase of claim 18, wherein said model endopeptidase
2 comprises a subsequence having at least 70% amino acid sequence identity to an amino acid
3 sequence of Figure 1.

1 20. The endopeptidase of claim 18, wherein said model endopeptidase is
2 encoded by a nucleic acid sequence that hybridizes under highly stringent hybridization
3 conditions to a nucleic acid encoding a polypeptide comprising an amino acid sequence of
4 Figure 1, wherein the hybridization reaction is incubated at 42°C in a solution comprising
5 50% formamide, 5x SSC and 1% SDS, and washed at 65°C in a solution comprising 0.2x
6 SSC and 0.1% SDS.

1 21. The endopeptidase of claim 18, wherein said one or more candidate
2 amino acid positions is selected from P129, E156, S191, G166, and G127.

1 22. The endopeptidase of claim 18, wherein said one or more candidate
2 amino acid positions is P129 and said point mutation is a glycine or alanine substitution.

1 23. The endopeptidase of claim 18, wherein said one or more candidate
2 amino acid positions is E156 and said point mutation is an arginine substitution.

1 24. The endopeptidase of claim 18, wherein said one or more candidate
2 amino acid positions is E156 and said point mutation is a lysine substitution.

1 25. The endopeptidase of claim 18, wherein said one or more candidate
2 amino acid positions is P129 and E156, wherein said point mutation is glycine at p129 and
3 arginine at E156.

1 26. The endopeptidase of claim 18, wherein, before step (a), said one or
2 more candidate amino acid positions are identified by a method comprising:

1 27. An isolated nucleic acid encoding a endopeptidase which site-
2 specifically cleaves a peptide bond of a post-translationally modified polypeptide at a site of
3 post-translational modification and which comprises one or more point mutations at one or
4 more amino acid positions within the endopeptidase active site,

5 wherein said isolated nucleic acid hybridizes under highly stringent
6 hybridization conditions to a nucleic acid sequence of Figure 2, wherein
7 the hybridization reaction is incubated at 42°C in a solution comprising
8 50% formamide, 5x SSC and 1% SDS, and washed at 65°C in a solution
9 comprising 0.2x SSC and 0.1% SDS.

1 28. An expression vector comprising the nucleic acid of claim 27.

1 29. A host cell transfected with the vector of claim 28.

1 30. An isolated nucleic acid encoding a endopeptidase which site-
2 specifically cleaves a polypeptide backbone amide bond of a post-translationally modified
3 polypeptide at a site of post-translational modification and which comprises one or more
4 point mutations at one or more amino acid positions within the endopeptidase active site,
5 wherein said isolated nucleic acid comprises a subsequence having at least
6 70% nucleic acid sequence identity to a nucleic acid sequence of Figure 2.

31. An expression vector comprising the nucleic acid of claim 30.

1 32. A host cell transfected with the vector of claim 30.